Novel insight in the life cycle of Torymus sinensis, biocontrol agent of the chestnut gall wasp

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Torymus sinensis Kamijo (Hymenoptera: Torymidae) is a biological control agent of the chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae). It is reported in the literature as univoltine, but in NW Italy it exhibits a prolonged diapause mainly as late instar larva. Diapause is extended for 12 months, and adults emerge in April as usual, showing a two-year life cycle. 2nd year emergence individuals are able to mate, and the presence of mature eggs was confirmed in females which parasitised fresh chestnut galls, showing the same parasitism behaviour as 1st year emergence individuals. Both sexes of 2nd year emergence individuals proved to be smaller than the univoltine ones according to ovipositor sheath length, pronotum width, and hind tibia length. Proving evidence of the extended diapause plays an important role for the establishment of *T. sinensis* especially in the first years after its release. Future studies are needed to clarify the factors which trigger off this response.

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Introduction

The alternation of active and dormant stages is an important trait of many invertebrate animals, including arthropods, affecting several aspects of their life cycles such as duration, phenology, and flexibility (Belozerov 2008). Indeed, many insects undergo diapause periods to get through adverse conditions in seasonal environments. Environmental conditions, principally temperature and photoperiod, activate the different steps of diapause induction, and influence its maintenance and termination; moreover, other aspects like food availability and quality, type and physiological status of the host, population density, are also involved in this event (Leather et al., 1993; Velarde et al., 2002). In fact, diapause can evolve as bet-hedging mechanism, occurring wherever there is a temporal variation in the suitability of the environment; this variation may be caused by temporally varying levels of parasitism, and it usually occurs in populations whose seasonal resources fluctuate unpredictably in abundance and availability (Ringel et al., 1998; Moraiti et al., 2012).

In some individuals of an insect population, such dormancy may be extended for more than one year and prolonged during the favourable season; this phenomenon is termed “prolonged diapause” (Waldbauer 1978; Hanski 1988). Prolonged diapause, by spreading adult emergence over time, allows the insect to overcome unpredictable environmental changes, allowing to some progeny to be ready for reproduction under better conditions (Corley et al. 2004). For this reason, for many insect species, this strategy is thought to protect demographic and genetic resources in fluctuating environments (Suez et al. 2013). On the other hand, the extension of the diapause period may be costly in terms of reproductive success, as dormant specimens could die before emergence (Soula and Menu 2003). Moreover, although metabolism is maintained at low rates during diapause (Lees 1955), dormant insects undergo continuous resource consumption, which may significantly affect fitness when diapause lasts for a long time (Matsuo 2006).

One of the insect models that has been studied for prolonged diapause is that of host-parasitoid systems. Parasitoids may undergo extended diapause in order to stay in synchrony with their hosts, in addition to maintaining the population during unfavourable conditions (Doutt et al. 1976).

Although many studies have been conducted, the effect of prolonged parasitoid diapause on the stability of interactions with the host is still unclear (Ringel et al. 1998; Corley et al. 2004).

Torymus sinensis Kamijo (Hymenoptera: Torymidae), native to China, is an exotic parasitoid of the Asian chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), a globally invasive pest of chestnut (*Castanea* spp). It was released as a biocontrol agent in Japan in 1975, in Georgia (USA) in the late 1970s, and in Italy in 2005 (Moriya et al. 2003; Cooper and Rieske 2007, 2011; Quacchia et al. 2008). It is phenologically well synchronised with its host and in all cases after its release is able to disperse successfully alongside *D. kuriphilus* by expanding its population, reducing shoot infestation rates below the tolerable damage threshold, and significantly containing gall wasp outbreaks.

T. sinensis is reported in the literature as univoltine like its host, predominantly reproducing amphigonically. Female lays eggs into newly formed galls, usually one egg per host larva. Under natural conditions, multiple eggs per host larva have been observed in a single chamber, but only one larva could grow up because of cannibalism among hatched young larvae (Piao and Moriya 1999). After hatching, the larva feeds externally on the mature host larva until pupation, which occurs during late winter. Adult wasps emerge from the withered galls of the chestnut gall wasp in the spring, synchronous with sprouting of chestnut trees and also with the appearance of *D. kuriphilus* galls (Moriya et al., 2003; Quacchia et al., 2008; EFSA Panel on Plant Health 2010; Cooper and Rieske, 2011).

In order to monitor the success of *T. sinensis* biocontrol activity in NW Italy, in 2012 withered galls were dissected after *T. sinensis* emergence to evaluate the number of unemerged specimens; during dissection, the presence of live *T. sinensis* larvae was revealed, highlighting a new aspect of the life cycle of this parasitoid. On the basis of this finding, the frequency of prolonged diapause in populations of *T. sinensis* was investigated in this area.

Materials and methods

Collection and dissection of the galls

In order to study the frequency of prolonged diapause of the parasitoid *T. sinensis*, investigations were carried out in 2013 in Cuneo province (NW Italy) where the parasitoid was first released in 2005 and then successfully established, forming stable populations. A total of five sampling sites were chosen. The sites were located in the municipalities of Boves (44°19’06’’N, 7°33’18’’E; 638 m asl), Caraglio (44°24'31,74"N, 7°24'05,98"E; 654 m asl), Cuneo (44°22'18,97"N, 7°33'51,81"E; 540 m asl), Peveragno (44°18’57’’N, 7°35’08’’E; 716 m asl), Robilante (44°18’14’’N, 7°31’09’’E; 775 m asl) (Fig. 1). Five naturally growing chestnut trees were randomly chosen at each site, and for each tree 200 galls that had formed during the previous year were randomly collected (20 galls x 10 branches) on the crown of the plant both during winter (February) and summer (June). Half of the winter-collected galls were individually isolated in plastic vials (120 mm in height by 25 mm in diameter) and kept in outdoor conditions until *T. sinensis* emergence. The number of *T. sinensis* adults emerging per gall was recorded, and the galls were then dissected. The remaining galls were stored in rearing cardboard boxes in outdoor conditions until the emergence of the adults. Summer-collected galls were divided in two subsets as well: half were immediately dissected, and half were stored until adult emergence as described above (Fig. 2).

Dissection was conducted using a stereomicroscope with the aid of a scalpel. The number of cells per gall was recorded, as well as the number of live larvae, pupae and/or unemerged adults.

Five newly emerged diapausing females and males, less than 24 h old, unfed and naïve, were isolated in a Petri dish containing dry filter paper (one female and one male per each dish, 12 mm in diameter) and their behaviour was observed to verify if they were able to mate and lay eggs. All mated females were then individually isolated in a Petri dish as described above containing a fresh unparasitised chestnut gall. Experiments, carried out under laboratory conditions (24±2°C, 60%RH), lasted 1 h or terminated when mating or oviposition occurred. Parasitised galls were then dissected using a stereomicroscope and the presence of eggs was recorded.

Twenty adults (ten males and ten females) were killed upon emergence with ethyl acetate and the pronotum width (maximum width), the hind tibia length, and the ovipositor sheath length (mm) were recorded, comparing 1st and 2nd year emergence *T. sinensis*. Measurements were taken using a Leica MZ16A stereomicroscope (50x magnification) with the software LAS version 3.7.0.

Identification of Torymus sinensis larvae

All the larvae and pupae found in the dissected galls were morphologically identified by comparison with the voucher specimens deposited at the DISAFA-Entomology laboratory. Furthermore, a sample of *T. sinensis* larvae (five larvae per each site and season), pupae (five pupae per each site and season, or all the pupae when fewer than five were found, with the exception of Robilante), and adults emerging in the second year (five males and five females per each site and season, or all the insects when fewer than five were found) were submitted to DNA extraction and then sequenced for the cytochrome oxidase I (COI) gene following Kaartinen et al. (2010) to confirm their morphological identification.

Statistical analyses

The number of individuals with extended diapause was referred to the total number of gall cells calculated within dissections in each year and site. After testing for homogeneity of variance (Levene test, $P<0.05$), data were analysed by Student's t-tests ($P<0.05$) to compare records obtained in different collection periods or by one-way analysis of variance (ANOVA) followed by Tukey test ($P<0.05$) to compare sites. To assess the sex ratio of emerged adults and diapausing pupae, χ^2 tests were performed ($P<0.05$). All analyses were performed using the software SPSS version 20.0 (SPSS, Chicago, IL).

Results

A total of 10,000 galls (2 collections x 5 sites x 5 trees x 200 galls) were collected at all the sampling sites. The number of cells per gall ranged from 3.563±0.069, recorded at the site of Peveragno, to 4.398±0.085, observed at the site of Cuneo, with an average of 3.851± 0.026.

The average number of 1^{st} year *T. sinensis* emerging per 100 cells in winter-collected galls was 85.37. Males were significantly more abundant than females (χ^2 test: df=1; χ^2=47.297; P<0.05), representing 54.91% of emerged adults, while females were 45.09%.

Dissected galls

Overall, considering the winter-collected galls, 90.70% of *T. sinensis* emerged in the first year, whereas the 2.56% remained inside the galls and the 6.72% died (Table 1). Gall dissection revealed an extended diapause at the larval and/or pupal stage occurring at all sites. Diapause rates related to such stages had significantly higher levels in galls collected in June than in winter-collected galls (Student's t-test: df=48; t=5.066; P<0.05). Moreover, the recorded number of dead parasitoids inside the galls was significantly lower in summer-collected galls (Student's t-test: df=48; t=6.845; P<0.05). Considering the totality of dissected galls (winter and summer collections) in single sites, a variability was observed (ANOVA: df=4, 45 ; F=6.568; P<0.05). According to the Tukey test, Caraglio showed a higher incidence of diapausing specimens than in all other localities.

Both larvae and pupae were found from galls collected at all sampling sites and periods, with the exception of winter-collected galls from Robilante, where only larvae were observed. However, diapausing larvae were always definitely more frequent, representing more than 80% of the individuals detected. Among pupae, a higher number of males than females was reported, with a mean of 0.22±0.05 male pupae and 0.12±0.04 female pupae per gall but no significant differences were detected (χ2 test: df=1; χ2=0.004; P=0.951). Nontheless in Peveragno we found more female than male pupae (χ2: df=1; χ2=0.03; P=0.873).

Stored galls

From the galls stored until 2014, adult parasitoids emerged in the spring of the second year, simultaneously with the emergence of univoltine adults. The average number of 2^{nd} year *T. sinensis* emerging per 100 cells was 0.37±0.06 for winter-collected galls and 1.05±0.22 for summer-collected galls (Student's t-test: df=48; t=2.835; P<0.05). Among the different sites, considering the totality of dissected galls (winter and summer collections) differences in parasitoid emergence after two years were observed (ANOVA: df=4, 45; F=7.921; P<0.05). The Tukey test showed that Robilante and Boves had the highest rates. Overall, we found a significantly higher number of males than females (χ^2 test: df=1; χ^2=53.1; P<0.05); males represented 80.46% of emerged adults, whereas females represented 19.54%.

Diapause lasted one year; in fact, from all the stored galls from which *T. sinensis* adults emerged, dissection during summer 2014 did not show any live larvae or pupae continuing their cycle. However, a mean of 7.81±0.05 and 1.07±0.14 dead specimens per 100 cells were found for winter-collected and summer-collected galls, respectively (Student's t-test: df=48; t=12.355; P<0.05). Hence, for winter-collected galls, we observed that 91.34% of *T. sinensis* emerged after one year, whereas 0.39% emerged after two years and 8.27% died (Table 1). Generally the emergence rates of diapausing individuals were lower than the diapause rates that we detected based on gall dissection in 2013; on average, the number of 2nd year emergence adults represented 26.06% of larvae and pupae that we observed in dissected galls. Conversely, considering only summer-collected galls from the sites of Boves and Robilante, the number of emerged adults from stored galls was higher than that recorded for juveniles in dissected galls.

All the newly emerged diapausing adults were able to mate and females laid eggs in fresh unparasitised chestnut galls. The average number (±SE) of eggs recorded per gall was 1.20±0.270.

Measurement of ovipositor sheaths length, pronotum width and hind tibia length (mm) in 1st and 2nd year emergence *T. sinensis* showed striking differences for all values (Table 2). The length of the ovopositor sheath was significantly shorter in 2nd year emergence females, with an average measure of 1.420±0.046 (Student's t-test: df=8; P<0.05; t=3.393). Also, the pronotum width differed significantly between 1st and 2nd year emergence adults in both males (Student's t-test: df=18; P<0.05; t=5.915) and females (Student's t-test: df=18; P<0.05; t=9.498). Similarly, significant differences were observed when measuring the hind tibia length of the two specimen groups, both in males (Student's t-test: df=18; P<0.05; t=6.301) and in females (Student's t-test: df=18; P<0.05;

t=9.471). Hence, 2nd year emergence adults were smaller than those that emerged in the first year in both sexes.

All the 406 morphologically analysed diapausing specimens were indeed *T. sinensis*. The cytochrome oxidase I gene obtained from a total of 75 specimens submitted to molecular identification was sequenced and sequences were compared with those in the National Center for Biotechnology Information (NCBI) sequence database. In all cases, a minimum of 99% similarity with *T. sinenisis*-related sequences was observed. The COI sequence of a specimen was deposited in the European Nucleotide Archive under the following accession numbers: LM651395.

Discussion

This study demonstrates that *T. sinensis* can undergo extended diapause, showing a two-year cycle. Second year emergence specimens were detected in the galls collected in all the surveyed sites. Since the time of the first release of *T. sinensis* in Piedmont in 2005, the number of *D. kuriphilus* host in the area has dramatically decreased, similar to the Japanese experience (Moriya et al., 1989), by limiting the food availability for the population of this monophagous wasp. A prolongation of diapause may be a response to food shortage (Hanski 1988). *T. sinensis* is known to be a specialist parasitoid, and the decline of the chestnut gall wasp may be one of the reasons why it exhibited an extended diapause. On the contrary, for the native parasitoid community commonly associated to gall wasps, extended diapause is a rare strategy probably because they are generalists and may shift on other available potential hosts in case of resource less predictable. Although we observed a lower incidence of extended diapause than in previous reports concerning beetles and wasps (Soula and Menu 2003; Mahdjoub and Menu 2008; Geisert and Meinke 2013; Suez et al. 2013), the percentage of individuals which prolong their diapause could grow over time in relation to *D. kuriphilus* availability. Even if extended diapause rates were always low, both gall dissection and 2nd year emergence adults showed a higher incidence of diapause when galls were collected in the summer. Also, mortality rates inside the galls were lower when collection was carried out in June, suggesting an influence of prolonged gall handling and storage on the successful life cycle completion of the insects.

Even taking into account the effect of gall collection, our results show that the number of diapausing larvae and pupae found in the galls was generally higher than the number of adults actually emerging in the second year. Furthermore, mortality rates detected by gall dissection in 2014 (after the emergence of 2^{nd} year emergence parasitoids) were always higher than those recorded in 2013. Additionally, individuals that emerged after a two-year diapause were significantly smaller than 1^{st} year emergence specimens. Individuals that express prolonged dormancy are in fact exposed to increased mortality and they postpone reproduction, both of which may result in fitness costs. Trade-offs in the allocation in the metabolic reserves between maintenance during dormancy and reproductive activity after dormancy have been reported in other wasp such as *Neodiprion swainei* and *N. sertifer* (Moraiti et al., 2012).

Taken together, these evidences highlight the cost of extended diapause, in terms of increased mortality and reduced growth, which is likely to be related to consumption of metabolic resources. Similar disadvantages have been previously reported; nonetheless, they are generally thought to be balanced by an increased chance of survival due to overcoming adverse conditions (Hanski 1988). Although reproductive costs for specimens with prolonged diapause have been reported as well (Soula and Menu 2003), we found that 2^{nd} year emergence *T. sinensis* females were able to mate and lay fertile eggs on chestnut galls.

The gall dissection highlighted a male-biased sex ratio among diapausing pupae, although without significant differences, confirming that for *T. sinensis* prolonged diapause is more common in males. These results are in agreement with Kraaijeveld and van Alphen (1995), who reported a similarly unbalanced sex ratio for individuals of the parasitoid *Asobara tabiada* Nees with extended diapause. Menu (1993) also detected a male-biased sex ratio for the chestnut weevil *Curculio elephas* Gyllenhaal emerging after three or four years. Although for *A. tiabida* the authors suggested that males underwent extended diapause more frequently than females (Kraaijeveld and van Alphen

1995), in the case of *C. elephas*, a higher emergence success in males than in females was observed (Menu 1993).

A striking variability in extended diapause rates was observed among different collection sites. According to gall dissection in summer 2013, the sites of Caraglio and Peveragno showed the highest extended diapause rates, whereas Boves and Robilante had the highest adult emergence rates after two years. Such variable results are likely to be due to the general unevenness of the extended diapause phenomenon itself. A detailed survey of microclimatic conditions in different localities could elucidate the possible influence of differences in temperature, relative humidity or rainfall, as commonly observed (Danks 1987). Furthermore, diapause intensity may be a response to winter warming, as suggested for the weevil *Exechesops leucopis* Wolfrum and the fruit fly *Ragholetis cerasi* Loew (Matsuo 2006; Moraiti et al. 2014).

This study contributes to the knowledge base needed to develop appropriate *T. sinensis* management strategies. In fact, extended diapause may have an adaptive value in protecting the population against the yearly fluctuation in food supply.

The presence of a parasitoid reservoir, consisting of larvae and pupae inside withered galls after the emergence of the univoltine population, is an important aspect for growers. In fact, throwing away or burning pruning discards after spring will eliminate diapausing individuals, reducing the wasp population emerging in the following spring. Therefore, all the plant material bearing galls (branches, suckers) can be cut away but not remove from the orchard at least for two years.

Even if the recorded diapause was low, this finding reveals its importance in sites where *T. sinensis* has not been released long or where its population is still at a low rate. Preserving the diapausing population will favour, in fact, the establishment of the parasitoid as well. Hence this novel insight in *T. sinensis*'s life cycle provides a decision-making tool to growers, playing an important role in chestnut orchard management in the first years after its release.

Prolonged diapause is a dynamic process and there is no doubt that the regulation of this strategy is extremely complex, making hard to speculate which are the factors that trigger off this response.

Unpredictable resources, adverse climatic conditions, and photoperiod induction may deeply influence growth and development in many insects (Schmidt et al., 2005; Moraiti et al., 2012; Ming-Xing et al., 2013).

Our investigations highlighted a new aspect of *T. sinensis* life cycle, but future studies are needed, carrying out surveys over a longer period of time at different parasitism rates, in order to clarify whether this event is actually increasing in relevance. Since in many insects geographically separated populations of the same species may show variations in diapause characteristics, it will be interesting to carry out investigations also in other European chestnut growing areas at different latitudes.

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Table 1 Parasitism levels of 1st and 2nd year emergence *T. sinensis* from galls sampled in different sites in NW Italy during 2013. Student's t-tests were performed on the data expressed as a mean of the five sites according to the collection month (Average line); dissected and stored galls were considered separately. In the average line within the same column values followed by the asterisk are significantly different (P<0.05), values followed by NS are not significantly different (P<0.05). ANOVA tests were carried out comparing values from different sites according to the collection month; dissected and stored galls were considered separately. Within the same column letters indicate significantly different values (Tukey test; P<0.05).

	Month	Site	1st year emergence / 100 cells ±SE[a]	Live larvae / 100 cells ±SE[b]	Live pupae / 100 cells ±SE[b]	Total diapausing *T. sinensis* / 100 cells ±SE[b]	Dead *T. sinensis* / 100 cells ±SE[b]	2nd year emergence / 100 cells ±SE[c]	Total *T. sinensis* / 100 cells ±SE
Dissected galls	February	Boves	82.90±0.97 A	2.17±0.60 AB	0.28 ±0.18 A	2.45±0.67 AB	4.63±0.48 A	-	89.98±1.12 A
		Peveragno	92.10±0.69 B	2.17±0.63 AB	0.21±0.08 A	2.38±0.71 AB	4.88±0.29 A	-	99.35±0.31 B
		Robilante	82.45±0.49 A	2.67±0.75 B	0.00 A	2.67±0.75 AB	5.31±0.90 A	-	90.43±0.97 A
		Cuneo	82.99±0.38 A	0.29±0.15 A	0.28±0.29 A	0.57±0.43 A	8.97±2.09 A	-	92.53±2.11 A
		Caraglio	82.53±0.81 A	3.36±0.21 B	0.51±0.18 A	3.87±0.36 B	7.58±0.88 A	-	93.98±0.93 A
		Average	**84.59±0.82 ND**	**2.13±0.30 ND**	**0.26±0.08 ***	**2.39±0.33 ***	**6.27±0.57 ***	**-**	**93.26±0.85 ND**
	June	Boves	-	1.10±0.37 A	0.27±0.15 A	1.37±0.52 A	1.26±0.21 A	-	-
		Peveragno	-	2.01±0.39 A	1.69±0.40 B	3.70±0.50 AB	1.97 ±0.66 AB	-	-
		Robilante	-	1.79±0.72 A	0.37±0.16 A	2.16±0.71 A	1.02 ±0.27 A	-	-
		Cuneo	-	2.35±0.39 A	0.30±0.09 A	2.65±0.46 A	2.53±0.50 AB	-	-
		Caraglio	-	4.75±0.75 B	0.60±0.27 A	5.35±0.68 B	3.54±0.33 B	-	-
		Average	**-**	**2.40±0.34 ND**	**0.65±0.15 ***	**3.05±0.37 ***	**2.07±0.26 ***	**-**	**-**
Stored galls	February	Boves	80.66±0.44 A	-	-	-	7.52±0.87 ABC	0.60±0.17 A	88.78±1.18 A
		Peveragno	93.75±0.69 C	-	-	-	4.84±0.92 A	0.43±0.11 A	99.03±0.35 B
		Robilante	77.79±1.13 A	-	-	-	11.08±0.96 C	0.45±0.15 A	89.31±0.98 A
		Cuneo	87.70±1.17 B	-	-	-	8.68±0.57 BC	0.16±0.05 A	96.54±1.63 B
		Caraglio	91.30±0.61 BC	-	-	-	6.94±0.79 B	0.20±0.04 A	98.44±0.53 B
		Average	**86.24±1.30 ND**	-	-	-	**7.81±0.54 ***	**0.37±0.06***	**94.42±1.01 ND**
	June	Boves	-	-	-	-	0.23±0.08 A	1.52±0.36 B	-
		Peveragno	-	-	-	-	1.12±0.28 AB	0.39±0.14 A	-
		Robilante	-	-	-	--	1.11±0.22 AB	2.75±0.39 B	-
		Cuneo	-	-	-	-	1.87±0.29 B	0.34±0.10 A	-
		Caraglio	-	-	-	-	1.04±0.29 AB	0.24±0.09 A	-
		Average	-	-	-		**1.07±0.14 ***	**1.05±0.22 ***	-

[a]Adult emergence was recorded in April 2013.

[b]Data were obtained by gall dissection carried out in June 2013 (dissected galls group) or in June 2014 (stored galls group)

[c]Adult emergence was recorded in April 2014.

Table 2. Average (±SE) ovipositor sheath length, pronotum width, and hind tibia length (mm) of 1st and 2nd year emergence *T. sinensis* (N=10). Student's t-tests were performed on data referred to males and females separately; within the same column values followed by the asterisk are significantly different ($P<0.05$).

Gender	Emergence year	Average ovipositor sheath length (mm) ±SE	Average pronotum width (mm) ±SE	Average hind tibia lenght (mm) ±SE
male	1st year	-	0.360 ±0.005 *	0.679±0.012 *
	2nd year	-	0.288±0.010 *	0.532±0.022 *
female	1st year	1.735±0.025 *	0.404±0.006 *	0.752±0.012 *
	2nd year	1.420±0.046 *	0.328±0.005 *	0.592±0.012 *

Figure 1 Location of the sampling sites in the province of Cuneo (black dots). The inset indicates the location of the Piedmont region in Italy

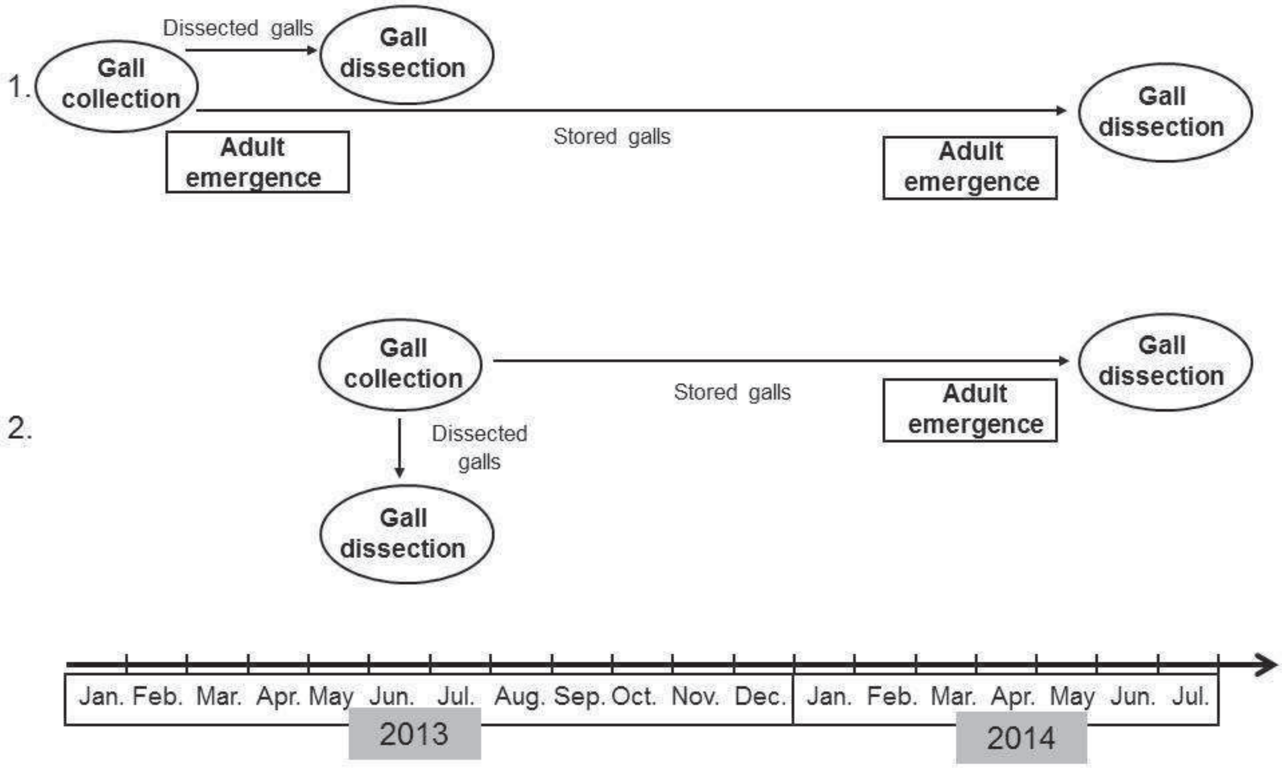

Figure 2 Experimental chart to evaluate the incidence of extended diapause in *T. sinensis* population in Piedmont, Italy, from winter-collected (1) and summer-collected (2) galls

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We wish to thank Johann Laimer who kindly provided the unparasitised chestnut galls used in the behavioural trials. We are also grateful to Michalis Bourellas, Marida Corradetti, Silvia Di Stefano, Cecilia Ferrara, Federica Fleury, and Valentina Tosi for their technical assistance.

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table 1

	Month	Site	1st year emergence / 100 cells ±SE[a]	Live larvae / 100 cells ±SE[b]	Live pupae / 100 cells ±SE[b]	Total diapausing *T. sinensis* / 100 cells ±SE[b]	Dead *T. sinensis* / 100 cells ±SE[b]	2nd year emergence / 100 cells ±SE[c]	Total *T. sinensis* / 100 cells ±SE
Dissected galls	February	Boves	82.90±0.97 A	2.17±0.60 AB	0.28 ±0.18 A	2.45±0.67 AB	4.63±0.48 A	-	89.98±1.12 A
		Peveragno	92.10±0.69 B	2.17±0.63 AB	0.21±0.08 A	2.38±0.71 AB	4.88±0.29 A	-	99.35±0.31 B
		Robilante	82.45±0.49 A	2.67±0.75 B	0.00 A	2.67±0.75 AB	5.31±0.90 A	-	90.43±0.97 A
		Cuneo	82.99±0.38 A	0.29±0.15 A	0.28±0.29 A	0.57±0.43 A	8.97±2.09 A	-	92.53±2.11 A
		Caraglio	82.53±0.81 A	3.36±0.21 B	0.51±0.18 A	3.87±0.36 B	7.58±0.88 A	-	93.98±0.93 A
		Average	**84.59±0.82 ND**	**2.13±0.30 ND**	**0.26±0.08 ***	**2.39±0.33 ***	**6.27±0.57 ***	**-**	**93.26±0.85 ND**
	June	Boves	-	1.10±0.37 A	0.27±0.15 A	1.37±0.52 A	1.26±0.21 A	-	-
		Peveragno	-	2.01±0.39 A	1.69±0.40 B	3.70±0.50 AB	1.97 ±0.66 AB	-	-
		Robilante	-	1.79±0.72 A	0.37±0.16 A	2.16±0.71 A	1.02 ±0.27 A	-	-
		Cuneo	-	2.35±0.39 A	0.30±0.09 A	2.65±0.46 A	2.53±0.50 AB	-	-
		Caraglio	-	4.75±0.75 B	0.60±0.27 A	5.35±0.68 B	3.54±0.33 B	-	-
		Average	**-**	**2.40±0.34 ND**	**0.65±0.15 ***	**3.05±0.37 ***	**2.07±0.26 ***	**-**	**-**
Stored galls	February	Boves	80.66±0.44 A	-	-	-	7.52±0.87 ABC	0.60±0.17 A	88.78±1.18 A
		Peveragno	93.75±0.69 C	-	-	-	4.84±0.92 A	0.43±0.11 A	99.03±0.35 B
		Robilante	77.79±1.13 A	-	-	-	11.08±0.96 C	0.45±0.15 A	89.31±0.98 A
		Cuneo	87.70±1.17 B	-	-	-	8.68±0.57 BC	0.16±0.05 A	96.54±1.63 B
		Caraglio	91.30±0.61 BC	-	-	-	6.94±0.79 B	0.20±0.04 A	98.44±0.53 B
		Average	**86.24±1.30 ND**	-	-	-	**7.81±0.54 ***	**0.37±0.06***	**94.42±1.01 ND**
	June	Boves	-	-	-	-	0.23±0.08 A	1.52±0.36 B	-
		Peveragno	-	-	-	-	1.12±0.28 AB	0.39±0.14 A	-
		Robilante	-	-	-	--	1.11±0.22 AB	2.75±0.39 B	-
		Cuneo	-	-	-	-	1.87±0.29 B	0.34±0.10 A	-
		Caraglio	-	-	-	-	1.04±0.29 AB	0.24±0.09 A	-
		Average	-	-	-		**1.07±0.14 ***	**1.05±0.22 ***	-

[a]Adult emergence was recorded in April, 2013.

[b]Data were obtained by gall dissection carried out in June 2013 (dissected galls group) or in June 2014 (stored galls group)

[c]Adult emergence was recorded in April, 2014.

table 2

Table 2. Average (±SE) ovipositor sheath length, pronotum width, and hind tibia length (mm) of 1st and 2nd year emergence *T. sinensis* (N=10). Student's t-tests were performed on data referred to males and females separately; within the same column values followed by the asterisk are significantly different (P<0.05).

Gender	Emergence year	Average ovipositor sheath length (mm) ±SE	Average pronotum width (mm) ±SE	Average hind tibia lenght (mm) ±SE
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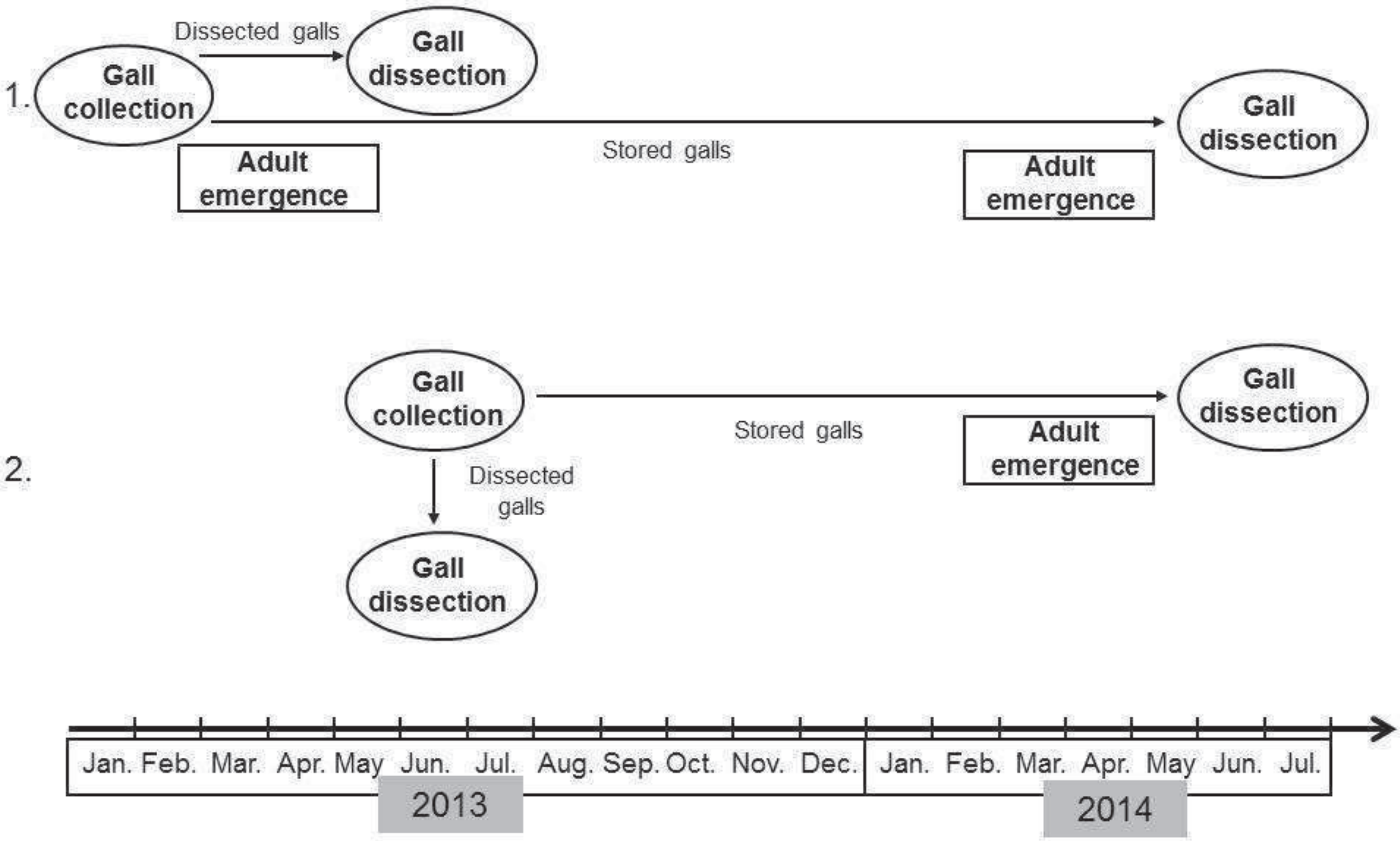
Figure 1

Figure 2



1.
Gall collection
Dissected galls
Gall dissection
Stored galls
Gall dissection
Adult emergence
Adult emergence
2.
Gall collection
Stored galls
Gall dissection
Adult emergence
Dissected galls
Gall dissection
Jan. Feb. Mar. Apr. May Jun. Jul. Aug. Sep. Oct. Nov. Dec.
2013
Jan. Feb. Mar. Apr. May Jun. Jul.
2014

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